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Assessment of drinking water quality at the tap using fluorescence spectroscopy

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Acronyms and abbreviations:

DOM	Dissolved organic matter
CDOM	Coloured dissolved organic matter
NOM	Natural organic matter
DOC	Dissolved organic matter
RSD	Relative standard deviation
TT	Triger threshold
HPC	Heterotrophic plate count
LoD	Limit of detection
EEMs	Excitation-emission matrices
PLS	Partial least squares
O-PLS	Orthogonal partial least squares

Abstract: Treated drinking water may become contaminated while travelling in the distribution system on the way to consumers. Elevated dissolved organic matter (DOM) at the tap relative to the water leaving the treatment plant is a potential indicator of contamination, and can be measured sensitively, inexpensively and potentially on-line via fluorescence and absorbance spectroscopy. Detecting elevated DOM requires potential contamination events to be distinguished from natural fluctuations in the system, but how much natural variation to expect in a stable distribution system is unknown. In this study, relationships between DOM optical properties, microbial indicator organisms and trace elements were investigated for households connected to a biologically-stable drinking water distribution system. Across the network, humic-like fluorescence intensities showed limited variation (RSD = 3.5 - 4.4%), with half of measured variation explained by interactions with copper. After accounting for quenching by copper, fluorescence provided a very stable background signal (RSD < 2.2%) against which a ~2% infiltration of soil water would be detectable. Smaller infiltrations would be detectable in the case of contamination by sewage with a strong tryptophan-like fluorescence signal. These findings indicate that DOM fluorescence is a sensitive indicator of water quality changes in drinking water networks, as long as potential interferents are taken into account.

Keywords: drinking water distribution, CDOM, natural organic matter (NOM), PARAFAC

1. Introduction

Between leaving a treatment plant and arriving at the consumer's tap, drinking water enters the distribution network where it resides for periods that typically range from hours to days. During this time, the drinking water may become contaminated via a range of processes. Microbial water quality can deteriorate in networks due to regrowth or entrainment of untreated water through damaged pipes, presenting potentially serious health risks to consumers (WHO 2014). In the United States during 1971-2006, around 10% of disease outbreaks caused by

unsafe drinking water have been attributed to deficiencies in the distribution network (Craun et al. 2010).

Microorganisms in drinking water distribution systems are either part of the indigenous community or enter the system where the pipe network integrity is compromised. Microbes living in soil pore-waters can be entrained through cracks in pipes and joints during negative pressure events (LeChevallier et al. 2003). Inside the pipes, heterotrophic bacteria utilise available organic substrate in the water as a source of carbon, nutrients and energy. Changing flow conditions in the network can also dislodge biofilms harbouring pathogenic species and create conditions that favour opportunistic species, potentially including pathogens (Manuel et al. 2007).

Obtaining rapid and affordable assessments of the microbial quality of drinking water is a famously intractable problem. Microbial indicator species including *Escherichia coli (E. coli)*, coliforms, *Enterococcus* spp. and total bacterial counts are frequently monitored as proxies for pathogens that are expensive or impractical to measure. Although the presence of *E. coli* and coliforms indicates contamination, their absence does not preclude the presence of other harmful organisms (Wu et al. 2011). Microbial biomass is typically measured via heterotrophic plate counts (HPCs), which quantifies bacteria that grow by consuming organic nutrients, i.e. a small fraction of total microorganisms in drinking water. The actual species quantified by HPC depends on cultivation medium, incubation temperature and incubation time (Allen et al. 2004). HPC levels are not regulated, although abundances above 500 cfu/ml are considered of potential concern, mainly due to interference with the analytical detection of total coliforms. HPC analyses typically take several days to implement assuming a well-equipped laboratory (Allen et al. 2004), hindering a rapid response to adverse measurements. Faster, cultivation-free methods for assessing microbial biomass exist, including adenosine tri-phosphate (ATP) and flow cytometry cell counts (FC), but these methods are still relatively complex to implement

and interpret, preventing their widespread use for monitoring distribution networks (Hammes et al. 2008, Van der Wielen and Van der Kooij 2010).

Dissolved organic matter (DOM) is a heterogeneous mixture of carbon-containing molecules present in all aquatic ecosystems. Globally, DOM plays a key role in carbon and nutrient cycling, and as a substrate for microbial growth, is one of the main risk factors promoting microbiological growth in distribution networks (Camper et al. 2003). DOM optical properties (absorbance and fluorescence) are widely used for studying changes in DOM composition and concentration (Murphy et al. 2013). Although neither spectroscopic technique necessarily directly measures the small bioavailable molecules consumed by heterotrophic bacteria, numerous studies have shown that optical measurements are nevertheless sensitive proxies of the wider DOM pool and track subtle changes in water quality (Stedmon et al. 2011, Stubbins et al. 2014). DOM fluorescence is a sensitive tracer of sewage contamination, correlating with *E. coli* abundances (Baker et al. 2015) and nutrients (Baker and Inverarity 2004) across systems.

Absorbance spectroscopy is frequently used to track the abundance of the coloured fraction of dissolved organic matter (CDOM) in drinking water treatment systems (Weishaar et al. 2003), including in online applications (Chow et al. 2008). Fluorescence spectroscopy is a much more sensitive technology, and additionally tracks compositional changes in DOM (Stedmon et al. 2011). However, studies of fluorescence in drinking distribution systems are very few. Hambly et al. (2010) surveyed houses serviced by two separate distribution systems (potable and recycled non-potable), and concluded that network cross-connections would be detectable from measuring fluorescence intensities at the tap. However it remain to be seen if organic matter fluorescence in drinking water networks is both stable and predictable enough to offer a sound baseline to identify contamination at point-of-use; and if the signal is correlated to microbial abundances and other chemical constituents in distribution systems.

Trace metals leached from pipe materials can potentially interfere with spectroscopic measurements of DOM in drinking water. In the presence of transition metals such as iron, copper and aluminium, metal-DOM complexes can form which absorb more strongly than un-complexed DOM while fluorescing less (Senesi et al. 1991, Yan et al. 2013). Corrosion by cast iron, galvanized iron and steel pipes are the main sources of iron in drinking water (WHO 2014). Copper is seldom used for municipal network pipes but is frequently used in household plumbing and fixtures. The suppression or quenching of DOM fluorescence by various metal ions has been studied in natural aquatic systems (Ryan 1982, Yamashita and Jaffe 2008) and wastewaters (Reynolds and Ahmad 1995). However, it is uncertain whether metals would interfere to any significant extent with DOM spectroscopic measurements in distribution systems where concentrations of DOM and metals are both low.

Chlorine is frequently applied at the end of drinking water treatment to limit regrowth and other microbial risks in the distribution network. In chlorinated networks, reactions between organic matter and chlorine break down large DOM molecules, decreasing aromaticity and fluorescence intensities and shifting fluorescence emission spectra (Beggs et al. 2009, Korshin et al. 1999). The effect of chlorine exposure on fluorescence intensities approximately follows an exponential decay curve, with rapid losses occurring at short reaction times (minutes to hours) followed by gradual losses at long exposures (Beggs et al. 2009). Chlorine could therefore be a confounding factor for comparing fluorescence measurements at the tap, particularly when chlorine doses are high and distribution times vary greatly.

In this study, relationships between DOM optical properties, microbial indicator organisms and trace element concentrations were investigated in a drinking water distribution network. The purpose was to assess whether DOM optical properties measured at the tap correlate with, and are potential surrogate indicators of, abundances of microbial indicator species. The study area had no reoccurring chemical or biological water quality issues, allowing determination of

baseline conditions in the network and thresholds to be established for recognising significant changes in water quality. Also, since there is much interest in using DOM optical properties for online water quality monitoring, we investigated whether trace elements sourced from within the pipe network interfere with DOM optical measurements at the tap. If significant interferences occur, this may seriously limit the interpretation of online DOM measurements if trace elements are not monitored at the same time.

2. Material and methods

2.1. Sampling and analytical methods

A municipal drinking water distribution network in central eastern Sweden was surveyed. The Gävle distribution system forms a 486 km network of predominantly iron and plastic (polyethylene) pipes. The plant receives groundwater, adjusts the pH with sodium hydroxide, and chlorinates before releasing it into the distribution system. Due to the groundwater source, the outgoing drinking water is moderately hard (calcium and magnesium hardness > 60 mgL^{-}). NaClO is dosed at 0.3-0.4 mgL^{-1} producing total chlorine in the outgoing water of 0.1-0.15 mgL^{-1}. Residual chlorine at the plant reacts rapidly with the NOM in the water to produce total chlorine concentrations (total chlorine = residual chlorine + chlorine demand) usually around 0.01-0.06 mgL^{-1} in the taps of buildings along the network. Thus the levels of free residual chlorine (FRC) in the network are much lower than is typical (>0.2 mgL^{-1}) to ensure a disinfection effect at the point of use (WHO 2014).

Drinking water samples were collected in winter (December 1-2, 2015) at 87 locations in houses and public buildings connected to the distribution system. Sampling locations were selected so as to encompass the entire range of water residence times experienced by households on the network (0.5-50 h). Water samples were obtained from taps in the kitchen or bathroom, after first flushing for 5 min. Replicate samples (n = 2) were collected at a subset of

sites (n = 10) to assess experimental and analytical reproducibility; these were both collected and measured completely independently of one another. Samples for microbial analyses and turbidity measurement were collected in sterile plastic (HDPE) bottles, DOM (dissolved organic carbon (DOC), absorbance, and fluorescence) samples in ashed amber glass bottles (DOM), and trace metal samples in acid-washed polyethylene tubes. DOM and metals samples were filtered on-site through pre-flushed, 0.45 μm cellulose acetate filters; lab tests indicated no measurable fluorescence after flushing with 120 mL of Milli-Q. Absorbance and fluorescence samples were analysed at Chalmers within 48 h of sampling. Trace metal samples were acidified to 1% v/v with high purity HNO_3 and analysed within 15 days. DOC samples were acidified to pH = 2 with high-purity HCl, stored at 4 ºC, and analysed within two months. Microbial samples and turbidity were analysed the following day at a commercial analytical laboratory (Eurofins).

In the laboratory, CDOM fluorescence and absorbance were measured in a 1-cm quartz cuvette using an Aqualog spectrofluorometer (Horiba Scientific). Excitation-emission matrices (EEMs) were obtained with 3 s integration time for excitation wavelengths 220-600 nm at 3-nm intervals and emission wavelengths of 240-800 nm at 2.3-nm intervals. Blank EEMs were acquired daily from ultra-pure water sealed in a quartz fluorometer cell and from MilliQ water. EEMs were spectrally corrected for instrumental biases and concentration effects according to established methods (Murphy et al. 2010). DOC was measured using a Shimadzu TOC-V_{CPH} carbon analyser, using the non-purgeable organic carbon (NPOC) method (EN 1484: 1997).

Concentrations of ten metals (Al, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS) using a Thermo Scientific iCAP Q spectrometer. The instrument was operated in standard mode for all elements, except for Fe and Ni which were analysed in kinetic energy discrimination (KED) mode with He as collision gas.

Microbial analyses were performed according to standard methods. Culturable heterotrophic bacteria counts were determined using the ISO HPC method 6222-M (ISO 6222: 1999) which involves incubation at 22°C for three or seven days. *E. Coli* and coliforms were enumerated by the IDEXX-Colilert method and *Enterococcus* spp. by the IDEXX-Enterolert method. Turbidity was measured using the SS-EN ISO 7027 method. In historical surveys of the distribution system (unpublished data), culturable microorganisms and slow-growing bacteria abundances were generally below 10 and 100 cfu/ml, respectively.

Chlorine was not measured during this survey; however, routine monitoring data are collected approximately monthly and indicate very low residual chlorine in the network. In samples collected immediately before and after this survey (n = 13), total chlorine was 0.03 mg/L (median) with a maximum of 0.04 mg/L at monitoring sites with distribution times of 8-41 hr; these numbers thus represent upper limits for chlorine residuals at the monitoring sites. These low values are consistent with long-term datasets archived with the Swedish Geological Survey (Vattentäktsarkivet 2016): in 2012-2015 total chlorine was typically below 0.05 mg/L (90th percentile = 0.08 mg/L, n = 400) at monitoring stations along this network. In this study, we use the chlorine reaction time as a proxy for chlorine residuals (Korshin et al. 2002). For all samples in this study, the chlorine reaction time exceeded 57 hours (2.4 days); therefore, it is expected that the Cl residuals at the time of fluorescence analysis were well below the upper limits indicated by the routine monitoring datasets.

To simulate the contamination of drinking water pipes by soil, and determine detection thresholds for observing the contamination, a serial dilution was performed of soil water added to drinking water. Soil was obtained from an urban area at a depth of approximate 1 m and its organic carbon content estimated by loss of ignition. The stock solution (2 g soil in 1 L of tap water) was mixed on a magnetic stirrer for 24 h then filtered through cellulose acetate (0.45 µm). The dilution series was prepared by diluting the stock solution using Milli-Q for 13

dilution factors between 1 and 1/200. Fluorescence and absorbance were measured the same day and DOC within three days.

2.2. Statistical methods

2.2.1. Relative standard deviation and detection limits

Independently-measured replicate samples were used to assess experimental and analytical error. Relative standard deviations (RSD = standard deviation/mean) are independent of scale and were used to compare how precisely different variables could be measured. Analytical detection limits were calculated as three times the standard deviation of triplicate blanks. Trigger thresholds (TT) were also determined, defined as the threshold for recognising a significantly elevated level of a tracer, for example due to its entrainment in the network via a cracked pipe (equation 1).

$$TT = \bar{x} + 3s \quad (1)$$

In equation 1, $\bar{x}$ and s are the average and standard deviation of measurements from samples collected across the whole network.

2.2.2. PARAFAC model

The fluorescence measurements generated a three dimensional dataset of EEMs (N = 87, after averaging data from experimental replicates). Within each EEM, the measured trilinear data can be modelled as the sum of a limited number of independently-varying fluorescence signals (Bro 1997). These independent signals can be quantified using the PARAFAC algorithm, which identifies the best-fitting excitation and emission spectra for each independent signal (termed a 'component') and its relative concentration in each sample. PARAFAC modelling was implemented on the corrected dataset using the *N-Way* and drEEM toolboxes for MATLAB according to established methods (Andersson and Bro 2000, Murphy et al. 2013).

Modelling performed with non-negativity constraints on all modes.

PARAFAC models were investigated with two to seven components, and split-half analysis, jack-knifing, and residual analysis used to select the most appropriate model. This process identified four independently-varying signals producing a four-component PARAFAC model and their intensities (F1-F4) in each sample (Murphy et al. 2013).

2.2.3. PLS model

Multivariate calibration is often used for process control when it is necessary to predict variables (Y) that are expensive or time-consuming to measure from a set of correlated variables (a matrix of X variables) that are measured more easily. In the context of drinking water monitoring, it would be desirable to predict microbial abundances from one or more easily-obtained chemical measurements. Partial least square (PLS) regression is often used for multivariate calibration since it performs well even when the number of predictor variables is high and some variables correlate with each other. When the PLS model is orthogonalised (O-PLS), all variation correlated to the response variable is compressed in the first latent variable, which greatly simplifies interpretation (Trygg and Wold 2002). In this study, slow-growing bacteria was the only microbial indicator detected at abundances that were high enough to be included in statistical analyses; all other microbial indicator species had high frequencies of non-detection. O-PLS was therefore used to predict slow-growing bacteria (Y) from twelve water quality variables (X, containing F_1, F_2, F_3, F_4, Al, Cu, Pb, Zn, Mn, Fe, DOC, absorbance at 254 nm (A_{254})). O-PLS regression was implemented using the PLS_Toolbox for MATLAB (ver. 8.1, Eigenvector Inc.). Before applying PLS, all predictor variables were transformed using the Cox-Box power transformation to improve adherences to a normal distribution; thereafter, each variable was autoscaled. Replicate measurements were averaged prior to modelling.

An iterative process was used to develop the PLS model. Initially, a model was created using all of the chemical data available with the aim to predict slow-growing bacteria abundances across all sites (N=87). Subsequently, this model was refined by removing the variables that had least influence on the model (lowest VIP). Still, this model had low predictive power and was not robust during cross-validation. It was then attempted to develop a model only for sites in the southeast parts of the distribution system (N=37) since these had generally higher bacterial counts and fewer non-detects. This also produced no robust patterns. Finally, a tentative model was developed for the southeast distribution system (N=31 after excluding five sites with low microbial abundances (< 25% percentile, <7 cfu/ml) and one site with high leverage on the model, and retaining only four parameters as predictor variables (F_4, Fe, A_{254}, Pb).

Metal complexation model

There are no established models for estimating metal-DOM complexation parameters from absorbance data. Two widely-used models for estimating the binding parameters of metal-ligand complexation from fluorescence data are the Ryan-Weber model (Ryan 1982) and modified Stern-Volmer model (Hays et al. 2004). Both assume 1:1 metal to ligand complex formation. The Ryan-Weber model assumes a linear relationship between the formed complex and fluorescence quenching, which may not reflect the full complexity of the binding mechanism (Hays et al. 2004). In the modified Stern-Volmer model, a nonlinear relationship is assumed, parameterized by a quenching constant (K_M) and an initial fraction (f) of fluorescence contributing to quenching. This Stern-Volmer model was used to estimate the binding parameters between PARAFAC components and copper in this study (equation 2).

$$\frac{F_0}{F_0 - F} = \frac{1}{f.K_M.C_M} + \frac{1}{f} \qquad (2)$$

In equation (2), F and F_0 are fluorescence intensities corresponding to the measured total

copper concentration C_M in samples containing copper, or in the absence of copper, respectively. K_M and f are the conditional stability constant and the fraction of initial fluorescence affected by metal binding. The K_M and f values were determined in this study from the relative fluorescence intensity of each component (equation 2) plotted against the inverse concentration of copper.

Effect of chlorine

Chlorine residuals decrease as a function of reaction time, and while rapid changes occur at short reaction times, at longer exposure times (e.g. a day or more), the rate of change can be assumed to be linear (Korshin et al. 2002). We defined the chlorine reaction time for a given sample as the time delay between chlorination at the plant and fluorescence analysis in the laboratory, which is assumed equal to the sum of its distribution time and the delay between sampling and analysis. To investigate whether chlorine exposure could have been a confounding factor in fluorescence measurements, general linear models were used to model fluorescence as a function of chlorine reaction time, both in the presence and the absence of a potential interaction with copper.

3. Results

3.1. Microbial and chemical water quality

3.1.1. Microbial indicators

Abundances of microbial indicator species were low or below detection limits across the entire distribution network. *Escherichia*. Abundances of *E. coli*, coliforms and *Enterococcus spp*. were below detection limits (< 1 per 100 ml) at all sites. Slow-growing bacteria abundances varied between 0-110 cfu/ml, and culturable microorganisms between 0-30 cfu/ml. Due to fewer non-detects, slow-growing bacteria was used as the primary indicator of microbial

abundance in all statistical analyses. Among paired replicate samples, the RSD of slow-growing bacteria abundance averaged 31% (Table 1).

3.1.2. Trace metals

All trace metals were detected at concentrations well below the health limits recommended by the World Health Organization (WHO 2011) (Table 1). No health limits exist for Fe, Al and Zn due to the very low concentrations of these metals in drinking water relative to levels that produce toxicological effects. Coefficients of variation for each metal are presented in Table 1. Variation among replicate measurements of Al, Pb, Cd and Cr was high (RSD > 50%), and concentrations were near the analytical detection limits. For all other trace metals, RSD was below 17%.

Table 1. Water quality parameters in the drinking water distribution network. • = no data, - = no limit.

Parameter	Min [1]	Max [1]	Median [1]	RSD across sites [1] (%)	RSD between replicates (%)		Outgoing [2]	LoD [3]	Heath limit [4]
					Median	Max			
Fe (µg/l)	1	20	4	75	7	17	< 20	0.74	-
Al (µg/l)	0.50	8	2	47	14	79	< 10	1.72	-
Cu (µg/l)	10	500	50	87	3	14	< 20	0.07	2000
Pb (µg/l)	<0.01	0.50	0.07	100	34	110	·	0.01	10
Zn (µg/l)	2	100	5	170	1	11	·	0.04	-
Mn (µg/l)	0.05	2	0.40	100	7	10	< 10	0.05	400
Ni (µg/l)	0.25	1.75	1.70	14	2	5	·	0.01	70
Cd (µg/l)	0.02	0.17	0.05	47	15	71	·	0.01	3
Cr (µg/l)	0.08	1	0.24	54	15	75	·	0.04	50
Mg (mg/l)	5.4	6.8	6.0	5	2	3	4	0.0005	-
F 1 (RU)	0.34	0.42	0.40	4	1	1.9	·	3 e-4	-
F 2 (RU)	0.25	0.31	0.29	4	1	1.4	·	<1 e-6	-
F 3 (RU)	0.19	0.23	0.22	4	0.7	1.1	·	4 e-4	-
F 4 (RU)	0.13	0.23	0.14	11	4	14	·	1 e-6	-

DOC (mg/l)	2.5	8.9	3.5	41	8	13	2.5	0.16	-
A_{254} (cm^{-1})	0.04	0.08	0.05	10	2	19	·	0.001	-
Slow-growing bacteria (cfu/ml)	< 1	110	17	100	25	94	·	1	-

[1] Data are from samples collected in houses along the network.

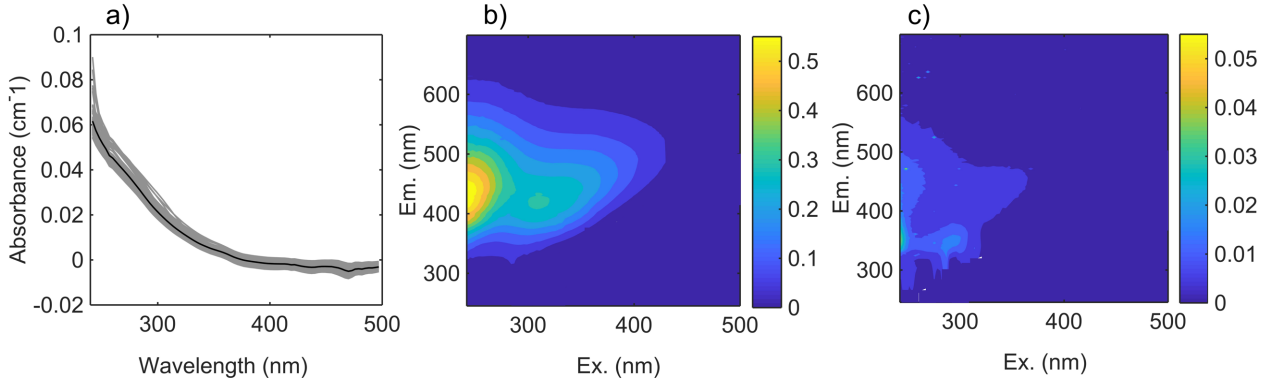
[2] Data reported by the WTP in the finished water leaving the plant.

[3] Analytical limits of detection (LoD) were determined from procedural blanks.

[4] Health limits are from WHO guidelines (WHO 2011).

3.1.3. *Turbidity,* DOM and DOC

Turbidity was low across the entire distribution network (0 – 0.27 FNU; average 0.14 FNU), and below detection (<0.1 FNU) at almost one third of sites. Spectroscopic measurements (absorbance and fluorescence) indicated low variability in the concentration and composition of optically-active DOM (Figure 1). Across the network, absorbance varied most at short excitation wavelengths (Figure 1a) and fluorescence at short excitation and emission wavelengths where protein-like fluorescence is observed (Figure 1b). Overall, absorbance was more variable than fluorescence (RSD = 10% for A_{254}, compared to 4% for humic-like peaks).



A four-component PARAFAC model explained 99.9% of the total variance in the fluorescence EEM dataset (Figure 2). Based on published interpretations of components with similar spectral properties (Coble 1996), the first three components (F_1: 314/408 nm F_2: 359/443 nm and F_3: 389/508) represent humic-like DOM and component 4 (F_4: 290/351) represents tryptophan-like DOM. Each fluorescence component was present at intensities exceeding the method detection limits in every sample.

Figure 1. Variation in optical properties across the distribution network. (a) Absorbance spectra (grey lines) compared to the average spectrum (black line); (b) Average fluorescence; (c) standard deviation of fluorescence; observe the change in scale.

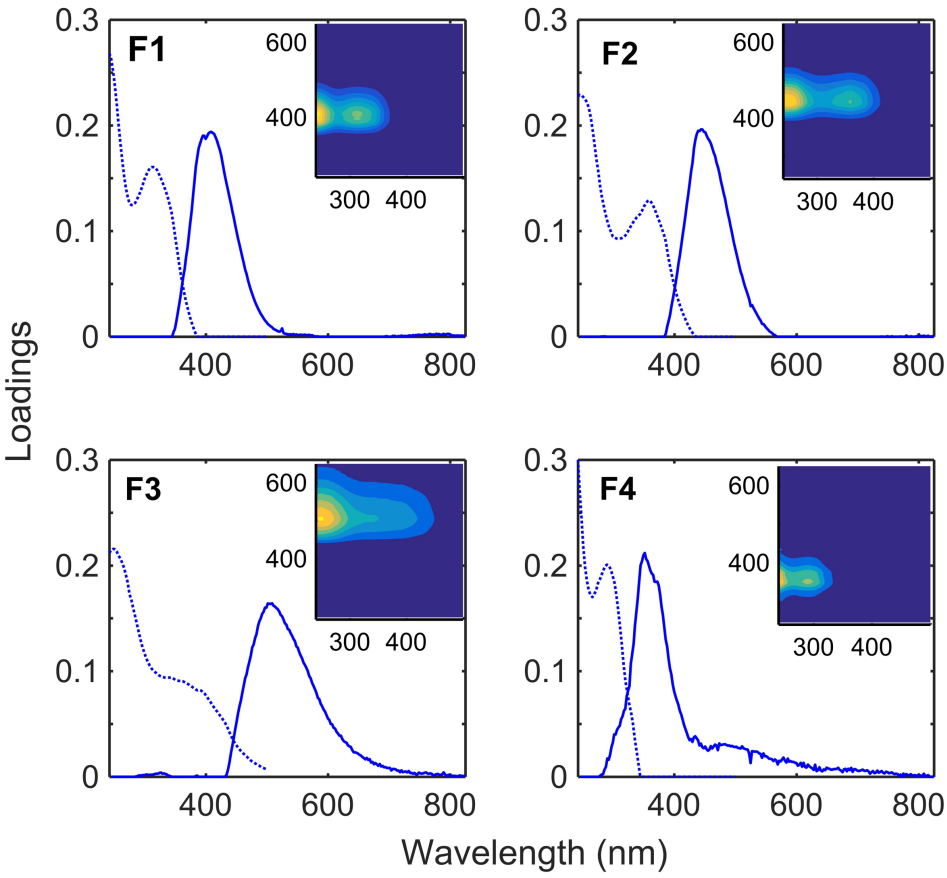


Figure 2. Spectral properties of four independently-varying fluorescent components (F1 - F4) identified in the drinking water network. Inserts show excitation wavelengths on horizontal axis and emission wavelengths on vertical axis.

Variation in fluorescence intensities could not be explained by differences in chlorine reaction time. In general linear models of fluorescence intensities regressed against chorine exposure time and/or copper, chlorine exposure time explained no more than 5% of total variability in any fluorescence component ($R^2 \leq 0.05$), compared to copper, which explained 56 - 63% of total variability for the humic-like components (F1 - F3) but less than 1% of the variability in the protein-like component (Supporting Information, Tables S1-S4).

DOC concentrations varied from 2.5 to 8.8 mg/L across the distribution network, with mean and median concentrations of 4.5 ppm and 3.5 ppm, respectively (Figure 3a). Two distinct DOC distributions could be observed; one with low DOC similar to DOC in the outgoing water from the plant (< 4 mg/L) and a second which was normally distributed with mean of approximately 7 mg/L. No geographical pattern could be detected that explained these two distributions. At the same time, the result could not be explained by contamination or by analytical error as samples were analysed in random order, and replicate samples spanned both distributions and differed by at most 16% (see the Supporting Information). Instead, this result indicates that an additional source of DOC was present either in the distribution network or else in the household pipe network, potentially including plastic piping and rubber seals in tap fittings.

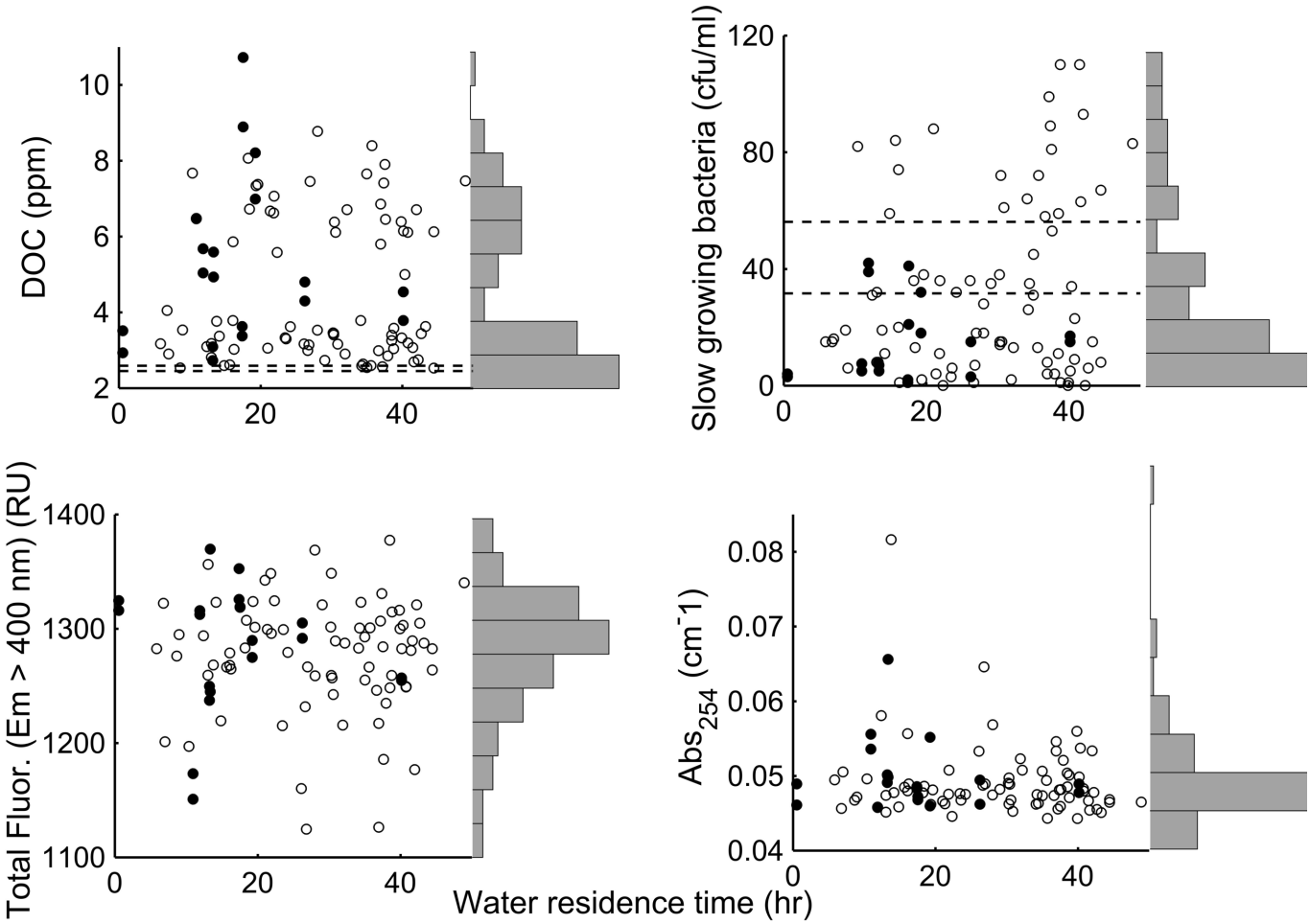


Figure 3. The distribution of DOC, slow-growing bacteria, total fluorescence (Em>400 nm) and A_{254} versus water residence time. Replicated samples are shown as filled circles. Histograms of the data are shown to the right of each plot

3.2. Effect of water residence time on water quality

No correlation was observed between water residence time and any of the individual chemical or microbial parameters measured in the distribution system (Figure 3). Also, no variation in chemical or microbial parameters could be attributed to the time of day when sampling took place. However, qualitative trends were observed for some parameters. When sites were divided in three groups having low (< 25th percentile, <7 cfu/ml), medium (25th -75th percentile, 7-40 cfu/ml) or high (>75th percentile, ≥ 40 cfu/ml) slow-growing bacteria abundance, sites with high slow-growing bacteria were often located in the southeast region of the distribution network (Figure 4a). Also, when divided in groups representing low (<17 h), medium (17-29 h), or high (≥ 29 h) water residence times, sites with long residence times mainly clustered in the same region (Figure 4b). In this southeast region, the average travel time was almost 10 hours longer than at other locations and the average slow-growing bacteria abundance was almost 1.7 times greater than the average for the remaining sites.

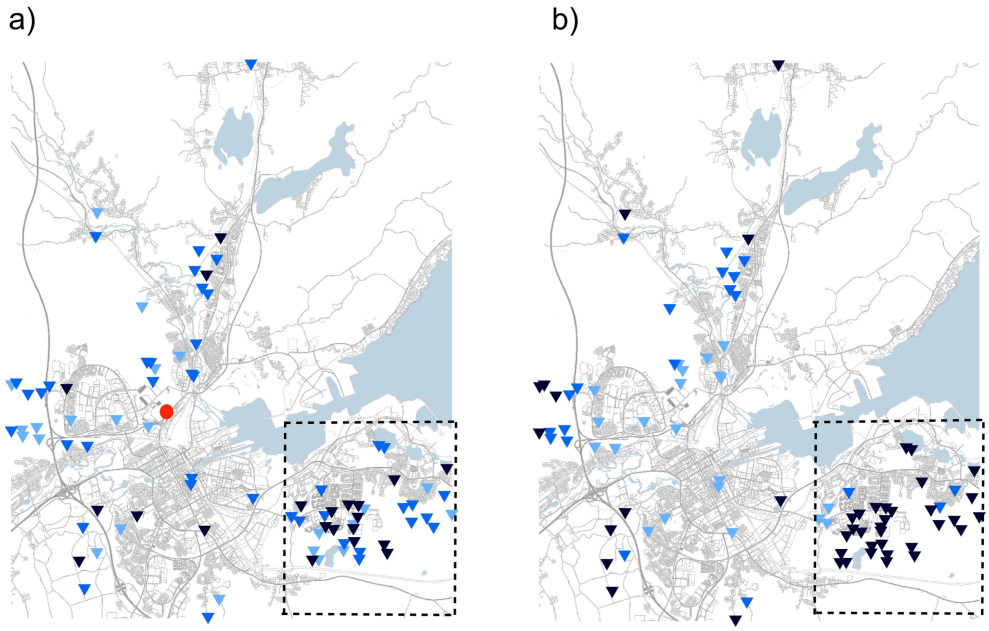


Figure 4. Spatial distributions of (a) slow-growing (7-day) culturable bacteria, and (b) water residence time, at houses in the Gävle distribution network. Sites are classified according to category ranging from high (darkest triangle) to low (lightest triangle). The water treatment plant (WTP) is shown as a red circle. Sites within the southeast network are shown enclosed in a dashed square.

3.3. Predicting microbial abundance from chemical variables

Four chemical variables (protein-like fluorescence F_4, A_{254}, Fe and Pb) were most useful for predicting slow-growing bacteria abundances in the southeast network where distribution times were longest. The PLS model of the southeast network explained 33% of the measured variation in slow-growing bacteria abundances and 61% of the measured variation in these four chemical parameters (RMSECV=2.7, RMSEC=2.1, N=31). Only tentative conclusions can be drawn from the model due to its restricted geographical range and relatively low predictive ability (R^2_{cv} = 35%). Along the only axis relevant to predicting microbial abundance, A_{254} and protein-like fluorescence were negatively correlated to slow-growing bacteria. This could occur if these autotrophic bacteria exerted top-down control on the abundance of protein-like fluorophores, or if protein-like fluorescence and bacterial abundance were both influenced by a third parameter but in opposite directions. Bacteria abundances were positively correlated with Fe, which is a potential food source for some types of autotrophic bacteria (Kirchman et al. 2000) but was not a significant ingredient in the HPC growth medium.

3.4. Copper and fluorescence/absorbance interaction

Copper concentrations were negatively correlated with each of the three humic-like fluorescence components, with Pearson correlation coefficients of 77 - 78% (Figure 5a, Table S5). At the same time, a positive correlation was observed between absorbance and copper concentrations (Figure 5b). Copper did not correlate with protein-like fluorescence. For each humic-like component, the modified Stern-Volmer model provided a reasonable fit to the fluorescence data, with copper explaining 37 - 49% of the measured variation in fluorescence intensities. This fit is illustrated for component F_2 in Figure 5. A better fit to the dataset was obtained using a linear-fit (R^2 = 56 - 62%) or a power-regression model (R^2 = 62 - 63%).

Assuming the Stern-Volmer model, the log K_m values for the three humic-like components in this study are comparable with values reported in earlier studies (Table 2).

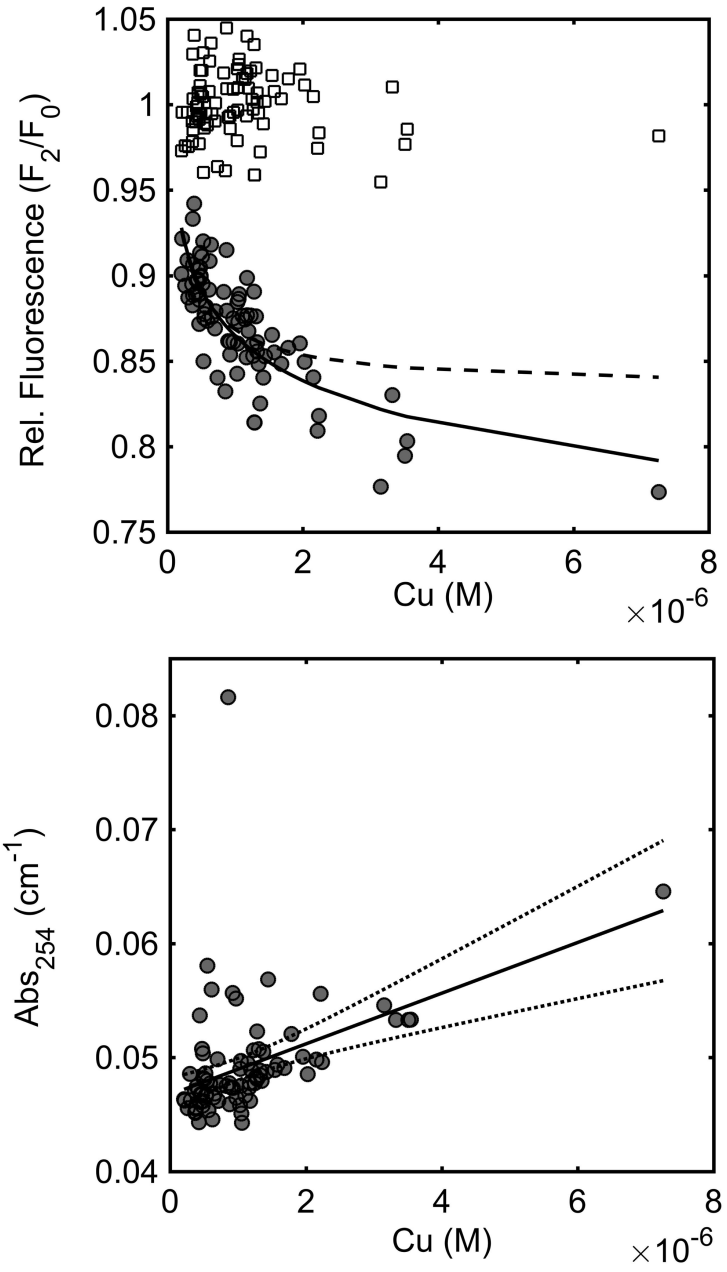


Figure 5. Correlations between DOM optical measurements and copper in the distribution network. (a) Relative fluorescence intensity (fluorescence / 0.33) of component F2 versus copper concentration. The solid line fits a power-regression model (R2 = 0.62), the dashed line a modified Stern-Volmer model (R^2 = 0.43). Closed circles show uncorrected data and open squares show corrected data assuming zero copper present under the power model. t (b) A_{254} versus copper concentration, R^2 = 0.2.

Table 2. Conditional stability constants (log K_M) and initial fraction, *f*, calculated using the modified Stern-Volmer model for humic-like fluorescence components in this study, compared to

Complexation parameters in this study			(Kirchman et al. 2000, Yamashita and Jaffe 2008)			(Xu et al. 2013)		
PARAFAC component	log K_M	*f*	PARAFAC component	Log K_M	*f*	PARAFAC component	log K_M	*f*
F_1	6.24	0.12	Component 1	4.91	0.54	Humic-like fluorescence	5.10	0.80
F_2	6.36	0.14	Component 6	5.45	0.30			
F_3	6.25	0.14	Component 2	4.81	0.61			

similar components in published studies.

The strong correlations between copper and fluorescence enabled the fluorescence data to be corrected for copper quenching by calculating what fluorescence intensities would have been in the absence of copper (i.e. at the intercept [Cu] = 0). Fluorescence intensities across the network were significantly less variable after copper correction, as illustrated by reduced coefficients of variation. Thus in the presence of copper, the coefficients of variation were between 3.6 - 4.1% (Table 3). After correcting the fluorescence data using the Stern-Volmer model, RSD decreased to 2.2 - 2.4%, while simple power or linear fitting reduced RSD even further to 1.3 - 2.2%.

3.5. Trigger threshold for detecting entrained contaminants

Trigger thresholds (*TT*) for observing significant changes in the levels of each chemical and microbial parameter in the distribution network are presented in Table 4. Relative trigger thresholds (TT_{rel} = threshold/mean) for humic-like fluorescence were low (1.1 - 1.3), reflecting high measurement precision and stable fluorescence intensities across the network, and indicating that a sample with fluorescence intensity only 10% higher than the network average

could be identified as being an outlier. Due to the highly variable DOC concentrations in the distribution system, soil water entrainment would have been undetectable on the basis of DOC. Trace metals had higher relative trigger thresholds than fluorescence (1.4 - 6.2) and would need to change by a larger relative amount before they would be distinguishable from natural variation. For slow-growing bacteria with *TT* around 3.9, a sample would not appear to be an outlier so long as microbial abundances were less than 390% of the network average (i.e. <120 cfu/mL in this study). Trigger thresholds could not be determined for other microbial indicator species, due to too many non-detects.

Table 3. Variation (RSD x 100%) in fluorescence intensities across the distribution network. Uncorrected data are compared with data corrected for quenching by copper using a modified Stern-Volmer model, a linear model, and a power-fit model.

Componen	Uncorrect	Stern-	Linear	Power
F_1:	3.6	2.2	1.9	1.9
F_2:	3.8	2.2	2.1	2.0
F_3:	4.1	2.4	1.3	2.2
F_4:	11	-	-	-

Table 4. Trigger thresholds for detecting outliers in the drinking water distribution network.

Parameter	Network mean	Network std. dev.	*TT*	*TT*/mean	Min.	Max.	Median
Fe (µg/l)	5.4	4.1	17.7	3.3	1	20	4
Al (µg/l)	2.8	1.3	6.7	2.4	0.50	8	2
Cu (µg/l)	69.1	61.4	253	3.7	10	500	50
Pb (µg/l)	0.08	0.09	0.35	4.4	<0.01	0.50	0.07
Zn (µg/l)	8.4	14.6	52.2	6.2	2	100	5
Mn (µg/l)	0.38	0.41	1.64	4.3	0.05	2	0.40
Ni (µg/l)	1.17	0.17	1.68	1.4	0.25	1.75	1.70
Cd (µg/l)	0.06	0.03	0.15	2.5	0.02	0.17	0.05
Cr (µg/l)	0.28	0.15	0.73	2.6	0.08	1	0.24
Mg (mg/l)	6.1	0.33	7.09	1.16	5.4	6.8	6.0
F_1 (RU) - Uncorrected - Corrected for [Cu]	 0.40 0.44	 0.014 0.009	 0.44 0.46	 1.1 1.06	 0.34 0.42	 0.42 0.46	 0.4 0.44
F_2 (RU) - Uncorrected - Corrected for [Cu]	 0.29 0.33	 0.010 0.007	 0.32 0.35	 1.1 1.06	 0.25 0.31	 0.31 0.34	 0.29 0.33
F_3 (RU) - Uncorrected - Corrected for [Cu]	 0.22 0.25	 0.009 0.005	 0.24 0.27	 1.1 1.07	 0.19 0.24	 0.23 0.26	 0.22 0.25
F_4 (RU)	0.14	0.02	0.19	1.3	0.13	0.23	0.14
DOC (mg/l)	4.6	1.9	10.34	2.24	2.5	8.9	3.5
A_{254} (cm^{-1})	0.05	0.005	0.06	1.20	0.04	0.08	0.05
Slow - growing bacteria (cfu/ml)	30.9	29.8	120.3	3.9	<1	110	17

4. Discussion

DOM optical properties are well-established water-quality tracers including for the treatment of drinking water (Murphy et al. 2011, Shutova et al. 2014, Stedmon et al. 2011). However, few DOM data have been reported from point-of-use in distribution networks, and it is unknown how much variability can be expected from spectroscopic measurements in stable systems. The network in this study had no known microbial issues, according to both this study and long-term (bimonthly) monitoring by the municipality. All houses sampled on the network produced samples with non-detectable levels of *E. coli,* enterococci and coliforms, together with low abundances of culturable bacteria (3-day and 7-day HPC). Globally, abundances of HPC bacteria vary widely in drinking water distribution systems (< 0.02-10^4 cfu/ml) depending on a range of factors including DOC and source water quality, treatment efficiency, distribution time, disinfection residual, and pipe condition (Allen et al. 2004). Elevated abundances of slow-growing bacteria were observed in this study in the section of the distribution network with longest water residence time; even so, concentrations were always below 110 cfu/ml and never approached levels for concern. Only weak correlations were observed between DOM optical measurements and HPC bacterial abundances, and only at locations where water residence time and microbial abundances were highest, suggesting that most observed variability was due to noise.

Due to a general lack of published reports on DOM in distribution networks, few data could be located for comparing to the current dataset. Tryptophan-like fluorescence was previously measured in Australian potable and recycled water networks (Hambly et al. 2010), where it was assessed as a tracer of cross-connections. Intensities in that study were measured in situ and reported in arbitrary units so cannot be directly compared with the current study, however the relative standard deviation of tryptophan-like fluorescence measurements in the Australian study was approximately three times higher than in the current study (RSD = 33% and 11%,

respectively). This is not surprising, because in-situ fluorometers are generally much less sensitive than benchtop fluorometers and produce noisier data. Additionally, tryptophan-like fluorescence depends on microbial activity (Moran et al. 2000), which would have been supressed by the winter temperatures in Sweden in comparison to Australian conditions.

For any water quality tracer, the more predictable its concentration within the distribution network, the easier it would be to detect contaminated water entrained through damaged pipes. In the current study, fluorescence was the most sensitive tracer among the suite of parameters measured due to high measurement precision and low variability across the network. The minimum amount of contaminated water detectable in practice depends upon the characteristics of the contaminant and the drinking water: as the difference between the two end-members increases, smaller entrainments can be detected. In the network, the average F_1 fluorescence intensity was 0.4 RU. If mixed with our soil water sample (F_1 = 1.4 RU), then the contaminated water would need to represent at least 4% of the total sample volume before it could be detected on the basis of fluorescence. After taking copper concentrations into account, a 2% infiltration of soil water would be detectable. Note that if the fluorescence signal of the entrained soil water decreases significantly due to interactions with copper, chlorine or other interferents, this would reduce overall sensitivity for detecting an infiltration event by fluorescence spectroscopy.

In comparison to humic-like tracers, tryptophan-like fluorescence exhibited higher measurement variation (RSD = 11%) even though its fluorescence was not quenched by copper. This variability could not be attributed to any other parameters monitored in this study, and probably reflects the higher lability as well as greater risk for contamination of this peak by trace amounts of organic matter. To provide comparable sensitivity to a humic-like tracer, tryptophan-like fluorescence would need to be at least ten times higher in the contaminated end-member than in the drinking water end-member. This would not be unusual if the contaminant were sewage, where tryptophan-like fluorescence intensities frequently exceed

drinking water levels by several orders of magnitude (Baker et al. 2015, Sorensen et al. 2015). *E. coli* concentrations and tryptophan fluorescence in environmental samples have been shown to correlate approximately linearly over a seven-log range (Baker et al. 2015). If so, tryptophan-like fluorescence could be a sensitive tracer of entrained sewage due to its low detection threshold coupled with high measurement precision.

Copper reduced the measured intensities of humic-like fluorescence in this study, as has been observed in other aquatic systems (Xu et al. 2013, Yamashita and Jaffe 2008). The main source of copper is likely to have been the corrosion of interior copper plumbing in the buildings (WHO 2011). Copper also represented around 0.7% of the pipe materials in the municipal distribution system. Humic-like fluorescence varied inversely with copper across sampling sites, with copper explaining 63% of the variation in fluorescence measurements under a linear regression model, compared with 43% for the modified Stern-Volmer model. The modest fit of the Stern-Volmer model may be due to relatively low copper concentrations in this study (Cu/DOC < 1/50) compared to the ranges typically studied (Cu/DOC < 1/25) (Reynolds and Ahmad 1995). The initial fraction of fluorescence contributing to quenching was also smaller than previously reported, possibly due to competition with calcium and magnesium ions for copper-binding sites (Ryan 1982). The suppression of DOM fluorescence by copper should thus be expected to vary between distribution systems, between sections of a network, and between nearby buildings on the network.

Water suppliers in some cities internationally have already made significant investments in online spectrophotometers for monitoring distribution systems, mainly using absorbance spectroscopy (Anon. 2013). In this study, absorbance was a less sensitive water quality tracer than was humic-like fluorescence; a 20% increase in A_{254} relative to the system average would be needed to trigger an outlier compared to a 10% increase for fluorescence, although absorbance exhibits a smaller natural range. For online instrumentation, however, the optimal

choice of online technology depends greatly on instrument cost and reliability. Also, although chlorine reaction time was not a confounding factor in this study due to a low chlorine dose and long exposure times, differential chlorine exposure could introduce artefacts that particularly affect online fluorescence monitoring, especially if chlorine doses are high and distribution times vary from hours to days.

For all parameters, since measurement variation increases over spatial and temporal scales, the detection threshold calculated in this study would almost certainly improve if comparing measurements at the tap with measurements at various local hubs located in network pipes, instead of with measurements from all households on the network. This would limit spatial and temporal variation, the effects of different household plumbing, and different degrees of chlorine exposure. Also, by comparing network hubs with one another, problems originating in the main pipe network could be isolated more easily.

5. Conclusions

- Organic matter fluorescence measurements in a functional and stable drinking water distribution system were well above detection limit and exhibited high measurement precision and low fluctuations across the network. Four independently varying fluorescence components were detected.
- Potential contamination in the distribution system that results in visible wavelength fluorescence exceeding the network average by 10% would be easily detectable.
- In-situ fluorometers should be capable of sensitively monitoring water quality changes in distribution systems between source and consumers, although issues related to reliability, sensitivity and calibration present technical hurdles worthy of further development and investigation.

- Trace metals can interfere with spectroscopic measurements in the distribution system and increase detection thresholds for observing significant changes in organic matter quality. It is therefore important to consider trace metals when investigating DOM fluorescence as a potential tracer of contamination in unfamiliar networks.

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